

PATENT COOPERATION TREATY

INTERNATIONAL APPLICATION NO. PCT/GB88/00649

NOTIFICATION OF ELECTION  
issued pursuant to PCT  
Rule 61.2

To:

United States Patent  
and Trademark Office  
Washington, D.C.

in its capacity as an elected Office

DATE OF MAILING OF  
THIS NOTIFICATION:  
18 April 1989 (18.04.89)

From:  
The International Bureau of WIPO  
1211 Geneva 20  
Switzerland

APPLICANT (NAME):

EKINS, Roger, Philip

INTERNATIONAL FILING DATE:

05 August 1988 (05.08.88)

PRIORITY DATE CLAIMED:

06 August 1987 (06.08.87)

The said election was made in the demand received by the International Preliminary  
Examining Authority on:

20 February 1989 (20.02.89)

J. Zahra  
(Authorized Officer)

10 Rec'd PCT 24 APR 89

Form PCT/IB/331 (June 1988)

BEST AVAILABLE COPY

18 Rec'd PCT/PTO

01 SEP 1988

PATENT COOPERATION TREATY

INTERNATIONAL APPLICATION NO. PCT/GB88/00649

NOTIFICATION TO THE DESIGNATED  
OFFICE OF RECEIPT OF  
RECORD COPY  
issued under PCT Rule 24.2(a)

To:

United States Patent  
and Trademark Office  
Washington, D.C.

in its capacity as a designated Office

DATE OF MAILING OF  
THIS NOTIFICATION:  
24 August 1988 (24.08.88)

From:

The International Bureau of WIPO  
1211 Geneva 20  
Switzerland

NAME(S) OF APPLICANT(S):

EKINS, Roger, Philip

INTERNATIONAL FILING DATE:

05 August 1988 (05.08.88)

PRIORITY DATE(S) CLAIMED:

06 August 1987 (06.08.87)  
10 February 1988 (10.02.88)

DATE OF RECEIPT OF RECORD COPY BY INTERNATIONAL BUREAU:

24 August 1988 (24.08.88)

J. Zahra  
(Authorized Officer)

PATENT COOPERATION TREATY

NOTIFICATION  
CONCERNING SUBMISSION  
OF PRIORITY DOCUMENT  
issued under Section 411 of  
the PCT Administrative  
Instructions

INTERNATIONAL APPLICATION No. PCT/GB88/00649

To:

HALE, Stephen, Geoffrey  
J. Y. & G. W. Johnson  
Furnival House  
14-18 High Holborn  
London WC1V 6DE  
ROYAUME-UNI

DATE OF MAILING OF  
THIS NOTIFICATION:  
23 September 1988 (23.09.88)

APPLICANT'S OR AGENT'S  
FILE REFERENCE:  
SGH/Case 9

From:  
The International Bureau of WIPO  
1211 Geneva 20  
Switzerland

INTERNATIONAL FILING DATE:  
05 August 1988 (05.08.88)

PRIORITY DATE(S) CLAIMED:  
06 August 1987 (06.08.87)  
10 February 1988 (10.02.88)

DATE OF RECEIPT OF PRIORITY DOCUMENT(S):  
23 September 1988 (23.09.88)

A copy of this Notification is being sent to each designated Office.

S. Taylor  
(Authorized Officer)

17 Rec'd PCT/PTO

02 JAN 1990  
PATENT COOPERATION TREATY

P 88/00649

TO

United States Patent  
and Trademark Office  
Washington, D.C.

FROM the INTERNATIONAL BUREAU of the  
WORLD INTELLECTUAL PROPERTY ORGANIZATION

NOTIFICATION CONCERNING  
DOCUMENTS TRANSMITTED

issued pursuant to PCT Article 13(1), 2(b),  
25(1)(a), (b), 36(3)(a), Rules 17.2, 66.7,  
23.1(b), and Administrative Instructions, Section 420

DATE OF MAILING by the International Bureau

18 December 1989 (18.12.89)

The International Bureau transmits herewith the following indicated  
types of documents and number thereof:

1. ☐ (number of) copies of international applications  
(Article 13(1), (2)(b)).
2. ☐ (number of) copies of documents in the files  
(Article 25(1)(a), (b)).
3. ☒ 1 (number of) copies of the ~~English translation of the~~  
international preliminary examination report  
~~and annexes~~ (Article 36(3)(a)).
4. ☐ (number of) copies of priority documents (Rules 17.2, 66.7).
5. ☐ (number of) copies of international applications due to  
lack of receipt of notification (Rule 23.1(b)).
6. ☐ (number of) copies of international applications and  
international search reports or declarations (Section 420).
7. ☐ other documents.

☐ Attached is a list identifying each document transmitted by the type  
of document it is, by the corresponding international application  
number and, if necessary, by other information.

This notification is sent to the above addressee in its capacity as:

- ☐ an International Searching Authority
- ☐ an International Preliminary Examining Authority
- ☐ a designated Office
- ☒ an elected Office

Mailing Address

WIPO  
34, chemin des Colombettes  
1211 Geneva 20  
Switzerland

Authorized Officer

J.-L. Baron

INTERNATIONAL APPLICATION  
UNDER THE  
PATENT COOPERATION TREATY  
REQUEST

THE UNDERSIGNED REQUESTS THAT THE PRESENT  
INTERNATIONAL APPLICATION BE PROCESSED  
ACCORDING TO THE PATENT COOPERATION TREATY

(The following is to be filled in by the receiving Office.)	
INTERNATIONAL APPLICATION No.	PCT/GB 88/00649
INTERNATIONAL FILING DATE:	05 August 1988 05 02 1988
(Stamp)	United Kingdom PCT International Application
Name of receiving Office and "PCT International Application"	
Applicant's or Agent's File Reference (indicated by applicant if desired)	SGH / Case 9

Box No. I TITLE OF INVENTION

DETERMINATION OF AMBIENT CONCENTRATIONS OF SEVERAL ANALYTES

Box No. II APPLICANT (WHETHER OR NOT ALSO INVENTOR); DESIGNATED STATES FOR WHICH HE/SHE/IT IS APPLICANT. Use this box for indicating the applicant or, if there are several applicants, one of them. If more than one person (includes, where applicable, a legal entity) is involved, continue in Box No. III.

The person identified in this box is (check one only): ☒ applicant and inventor\* ☐ applicant only

Name and address:\*\*

EKINS, Roger Philip,  
Department of Molecular Endocrinology,  
Middlesex Hospital Medical School,  
Mortimer Street,  
London, W1N 8AA,  
United Kingdom.

Telephone number:  
(including area code)

Telegraphic address:

Teleprinter address:

Country of nationality: United Kingdom

Country of residence:\*\*\* United Kingdom

The person identified in this box is *applicant* for the purposes of (check one only):

☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

Box No. III FURTHER APPLICANTS, IF ANY; (FURTHER) INVENTORS, IF ANY; DESIGNATED STATES FOR WHICH THEY ARE APPLICANTS (IF APPLICABLE). A separate sub-box has to be filled in in respect of each person (includes, where applicable, a legal entity). If the following two sub-boxes are insufficient, continue in the "Supplemental Box." (giving there for each additional person the same indications as those requested in the following two sub-boxes) or by using a "continuation sheet."

The person identified in this sub-box is (check one only): ☐ applicant and inventor\* ☐ applicant only ☐ inventor only\*

Name and address:\*\*

If the person identified in this sub-box is *applicant* (or *applicant and inventor*), indicate also:

Country of nationality:

Country of residence:\*\*\*

and whether that person is *applicant* for the purposes of (check one only):

☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

The person identified in this sub-box is (check one only): ☐ applicant and inventor\* ☐ applicant only ☐ inventor only\*

Name and address:\*\*

If the person identified in this sub-box is *applicant* (or *applicant and inventor*), indicate also:

Country of nationality

Country of residence:\*\*\*

and whether that person is *applicant* for the purposes of (check one only):

☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the "Supplemental Box"

\* If the person indicated as "applicant and inventor" or as "inventor only" is not an *inventor* for the purposes of all the designated States, give the necessary indications in the "Supplemental box."

\*\* Indicate the name of a natural person by giving his/her family name first followed by the given name(s). Indicate the name of a legal entity by its full official designation. In the address, include both the postal code (if any) and the country (name).

\*\*\* If residence is not indicated, it will be assumed that the country of residence is the same as the country indicated in the "Supplemental Box."

PCT 101

**Box No. IV AGENT (IF ANY) OR COMMON REPRESENTATIVE (IF ANY): ADDRESS FOR NOTIFICATIONS (IN CERTAIN CASES).** A common representative may be appointed only if there are several applicants and if no agent is or has been appointed. The common representative must be one of the applicants. The following person (includes, where applicable, a legal entity) is hereby/has been appointed as agent or common representative to act on behalf of the applicant(s) before the competent International Authorities:

Name and address, including postal code and country:

If the space below is used instead for an address for notifications, mark here

HALE, Stephen Geoffrey,  
J. Y. & G. W. Johnson,  
Furnival House,  
14-18 High Holborn,  
London, WC1V 6DE, United Kingdom.

Telephone number (including area code) 01-405-0356

Telegraphic address:

Teleprinter address: 27183 (JYJOHN G)

**Box No. V DESIGNATION OF GROUPS OF STATES OR STATES (1); CHOICE OF CERTAIN KINDS OF PROTECTION OR TREATMENT.** The following designations are hereby made (please mark the applicable check-boxes):

**Regional Patent**

☒ **EP European Patent(2):** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany (Federal Republic of), FR France, GB United Kingdom, IT Italy, LU Luxembourg, NL Netherlands, SE Sweden, and any other Contracting State of the European Patent Convention which has become party to the PCT after the issuance of this sheet (specify on dotted line):

☐ **OA OAPI Patent:** Benin, Cameroon, Central African Republic, Chad, Congo, Gabon, Mali, Mauritania, Senegal, Togo, and any other member State of OAPI which has become party to the PCT after the issuance of this sheet; if other OAPI title desired, specify on dotted line(3):

**National Patent (if other kind of protection or treatment desired, specify on dotted line(3))**

- |   |  |
|---|--|
| <input checked="" type="checkbox"/> AT Austria(3)                           | <input checked="" type="checkbox"/> KR Republic of Korea(3)        |
| <input checked="" type="checkbox"/> AU Australia(3)                         | <input type="checkbox"/> LK Sri Lanka                              |
| <input type="checkbox"/> BB Barbados  | <input type="checkbox"/> LU Luxembourg(3)                          |
| <input type="checkbox"/> BG Bulgaria(3)                                     | <input type="checkbox"/> MC Monaco(3)                              |
| <input checked="" type="checkbox"/> BR Brazil(3)                            | <input type="checkbox"/> MG Madagascar                             |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input type="checkbox"/> MW Malawi(3)                              |
| <input checked="" type="checkbox"/> DE Germany (Federal Republic of)(3)     | <input checked="" type="checkbox"/> NL Netherlands                 |
| <input type="checkbox"/> DK Denmark   | <input checked="" type="checkbox"/> NO Norway                      |
| <input checked="" type="checkbox"/> FI Finland                              | <input type="checkbox"/> RO Romania                                |
| <input checked="" type="checkbox"/> GB United Kingdom                       | <input type="checkbox"/> SD Sudan                                  |
| <input checked="" type="checkbox"/> HU Hungary                              | <input checked="" type="checkbox"/> SE Sweden                      |
| <input checked="" type="checkbox"/> JP Japan(3)                             | <input checked="" type="checkbox"/> SU Soviet Union(3)             |
| <input type="checkbox"/> KP Democratic People's Republic of Korea(3)        | <input checked="" type="checkbox"/> US United States of America(3) |

Space reserved for designating States (for the purposes of a national patent) which have become party to the PCT after the issuance of this sheet:

(1) The applicant's choice of the order of designations may be indicated by marking the check-boxes with sequential arabic numerals (see also the "Notes to Box No. V").  
(2) The selection of particular States for a European patent can be made upon entering the national (regional) phase before the European Patent Office (see also the "Notes to Box No. V").  
(3) If another kind of protection or a title of addition or, in the United States of America, treatment as a continuation or a continuation-in-part is desired, specify according to the instructions given in the "Notes to Box No. V".

**Box No. VI PRIORITY CLAIM (IF ANY).** The priority of the following earlier application(s) is hereby claimed.

Country (country in which it was filed if national application; one of the countries for which it was filed if regional or international application)	Filing Date (day, month, year)	Application No.	Office of Filing (fill in only if the earlier application is an international application or a regional application)
(1) GB	10 - 02 - 1988 10 February 1988	8803000	
(2) <del>[PCT]</del> [GB]	<del>6 - 08 - 1987</del> 6 August 1987	<del>[PCT/GB] 87/00558</del>	[GB]
(3)			

(Letter codes may be used to indicate country and/or Office of filing)

When the earlier application was filed with the Office which, for the purposes of the present international application, is the receiving Office, the applicant may, against payment of the required fee, ask the following:

☐ the receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the above-mentioned earlier application/of the earlier applications identified above by the numbers (insert the applicable numbers)

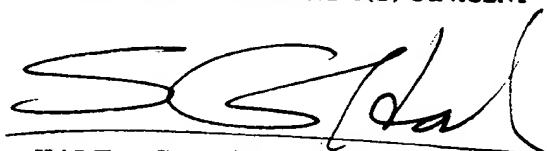
**Box No. VII EARLIER SEARCH (IF ANY).** Fill in where a search (international, international-type or other) by the International Searching Authority has already been requested (or completed) and the said Authority is now requested to base the international search, to the extent possible, on the results of the said earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

International application number or number and country (or regional Office) of other application: *EP number not known - see Case 7* International/regional/national filing date: *5 August 1988*

Date of request for search: *5 August 1988*

Number (if available) given to search request:

**Box No. VIII SIGNATURE OF APPLICANT(S) OR AGENT**

  
HALE, Stephen Geoffrey

If the present Request form is signed on behalf of any applicant by an agent, a separate power of attorney appointing the agent and signed by the applicant is required. If in such case it is desired to make use of a general power of attorney (deposited with the receiving Office), a copy thereof must be attached to this form.

**Box No. IX CHECK LIST (To be filled in by the Applicant)**

This international application contains the following number of sheets:

1. request	3	sheets
2. description	22	sheets
3. claims	3	sheets
4. abstract	1	sheets
5. drawings	1	sheets
<b>Total</b>	<b>30</b>	<b>sheets</b>

Figure number ..... of the drawings (if any) is suggested to accompany the abstract for publication.

This international application as filed is accompanied by the items checked below:

- ☒ separate signed power of attorney
- ☐ copy of general power of attorney
- ☐ priority document(s) (see Box No. VI)
- ☐ receipt of the fees paid or revenue stamps
- ☐ cheque for the payment of fees
- ☐ request to charge deposit account
- ☐ other document (specify)

(The following is to be filled in by the receiving Office)

- Date of actual receipt of the purported international application: 05 August 1988  
05 - 08 - 1988
- Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
- Date of timely receipt of the required corrections under Article 11 of the PCT:
- Drawings ☐ Received ☐ No Drawings

(The following is to be filled in by the International Bureau)

Date of receipt of the record copy: 24 AUGUST 1988 (24. 08. 88)

<b>IDENTIFICATION OF THE INTERNATIONAL APPLICATION</b>		<b>Applicant's or Agent's File Reference</b>																							
International Application No.  PCT/GB 88/00649		International Filing Date  5 August 1988 (05.08.88)																							
Receiving Office  UK Patent Office		Priority Date Claimed 6 August 1987 (06.08.87) 10 February 1988 (10.02.88)																							
Applicant (Name)  EKINS, ROger Phillip																									
<b>BASIS OF REPORT</b>																									
<p><b>1. AMENDMENTS AND/OR RECTIFICATIONS:</b> — The amendments and/or rectifications made before the International Preliminary Examining Authority in respect of the claims, the description, and/or drawings in the above-identified international application are annexed to this report.</p> <p>a. <input checked="" type="checkbox"/> This report has been established on the basis of the following application documents:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <input checked="" type="checkbox"/> the application documents as filed </td> <td style="width: 50%;"></td> </tr> <tr> <td style="vertical-align: top;"> <input type="checkbox"/> description, pages </td> <td style="vertical-align: top;">as originally filed</td> </tr> <tr> <td style="vertical-align: top;">description, pages</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;">description, pages</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;">description, pages</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;"> <input type="checkbox"/> claim(s) </td> <td style="vertical-align: top;">as originally filed</td> </tr> <tr> <td style="vertical-align: top;">claim(s)</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;">claim(s)</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;">claim(s)</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> <tr> <td style="vertical-align: top;"> <input type="checkbox"/> drawings, sheet/fig. </td> <td style="vertical-align: top;">as originally filed</td> </tr> <tr> <td style="vertical-align: top;">drawings, sheet/fig.</td> <td style="vertical-align: top;">filed with your letter of</td> </tr> </table> <p>b. <input type="checkbox"/> The amendments resulted in the cancellation of the following sheets: .....</p> <p>c. <input type="checkbox"/> This report has been established as if the amendments indicated on the extra sheet have not been made, since, for the reasons indicated, they have been considered to go beyond the disclosure as filed.</p>				<input checked="" type="checkbox"/> the application documents as filed		<input type="checkbox"/> description, pages	as originally filed	description, pages	filed with your letter of	description, pages	filed with your letter of	description, pages	filed with your letter of	<input type="checkbox"/> claim(s)	as originally filed	claim(s)	filed with your letter of	claim(s)	filed with your letter of	claim(s)	filed with your letter of	<input type="checkbox"/> drawings, sheet/fig.	as originally filed	drawings, sheet/fig.	filed with your letter of
<input checked="" type="checkbox"/> the application documents as filed																									
<input type="checkbox"/> description, pages	as originally filed																								
description, pages	filed with your letter of																								
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<input type="checkbox"/> claim(s)	as originally filed																								
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claim(s)	filed with your letter of																								
<input type="checkbox"/> drawings, sheet/fig.	as originally filed																								
drawings, sheet/fig.	filed with your letter of																								
<p><b>2. PRIORITY:</b></p> <p>a. This report has been established as if no priority has been claimed due to the failure to furnish within the prescribed time limit the requested:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <input type="checkbox"/> copy of the earlier application whose priority has been claimed. </td> <td style="width: 50%;"></td> </tr> <tr> <td style="vertical-align: top;"> <input type="checkbox"/> translation of the earlier application whose priority has been claimed. </td> <td></td> </tr> </table> <p>b. <input type="checkbox"/> This report has been established as if no priority has been claimed due to the fact that the priority claim has been found invalid.</p> <p>Thus, for the purpose of this report, the international filing date indicated above is considered to be the relevant date.</p>				<input type="checkbox"/> copy of the earlier application whose priority has been claimed.		<input type="checkbox"/> translation of the earlier application whose priority has been claimed.																			
<input type="checkbox"/> copy of the earlier application whose priority has been claimed.																									
<input type="checkbox"/> translation of the earlier application whose priority has been claimed.																									

\* Where replacement sheets are annexed to this report, a translation of these replacement sheets must be furnished to the elected Office within the time limit applicable under PCT Article 38(1).

**CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all.)**

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC<sup>4</sup>: G01N 33/543 G01N 33/78 33/76

**REASONED STATEMENT AS TO CLAIMS MEETING CRITERIA OF NOVELTY (N), INVENTIVE STEP (IS) AND INDUSTRIAL APPLICABILITY (IA); AND CITATIONS AND EXPLANATIONS SUPPORTING SUCH STATEMENT**

CLAIM NUMBER	STATEMENT (CRITERIA)	CITATIONS AND EXPLANATIONS
1-11	Yes(N, IS, IA)	<p>All claims meet the requirements of novelty, inventive step and industrial application. W088/01058, 84/01031 and GB 2099578 are acknowledged in the specification as prior art which does not teach or lead towards the specific V/K ratio of the invention.</p> <p>GB 2030290 and W086/01604 are merely illustrative of solid phase immunoassays. Clin. Chem is concerned with a specific mathematical treatment of the T4 binding system and does not lead towards the present invention.</p>

**NON-WRITTEN DISCLOSURES:**

Kind of Non-Written Disclosure	Date of Written Disclosure referring to the Non-Written Disclosure	Date of Non-Written Disclosure

**CERTAIN PUBLISHED DOCUMENTS 10**

Application/Patent	Date of Publication	Filing Date	Priority Date (Valid Claim)

### **CERTAIN DEFECTS IN THE INTERNATIONAL APPLICATION"**

The following defects in the form or contents of the international application have been noted:

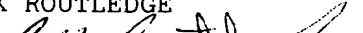
The claims are not in the two-part form as required by Rule 6,3 (b).

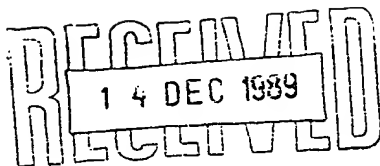
## CERTAIN OBSERVATIONS ON THE INTERNATIONAL APPLICATION 13

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description have been noted.

The preferred feature of Claim 11 should be made the subject of an appendant.

### CERTIFICATION

<p><b>Date Demand Submitted</b></p> <p>20 February 1989 (20-02-1989)</p>	<p><b>Date of Completion of the International Preliminary Examination Report</b></p> <p>4 December 1989 (04-12-89)</p>
<p><b>International Preliminary Examining Authority</b></p> <p>UK Patent Office</p>	<p><b>Signature of Authorized Officer</b></p> <p>B K ROUTLEDGE</p> 



# PATENT COOPERATION TREATY

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or Agent's File Reference
International Application No. PCT/GB 88/00649	International Filing Date 5 August 1988 (05.08.88)	
Receiving Office UK Patent Office	Priority Date Claimed 6 August 1987 (06.08.87) 10 February 1988 (10.02.88)	
Applicant (Name) EKINS, Roger Phillip		
BASIS OF REPORT		
1. AMENDMENTS AND/OR RECTIFICATIONS: — The amendments and/or rectifications made before the International Preliminary Examining Authority in respect of the claims, the description, and/or drawings in the above-identified international application are annexed to this report.		
a. <input checked="" type="checkbox"/> This report has been established on the basis of the following application documents:		
<input type="checkbox"/> the application documents as filed		
<input type="checkbox"/> description, pages	as originally filed	
description, pages	filed with your letter of	
description, pages	filed with your letter of	
description, pages	filed with your letter of	
<input type="checkbox"/> claim(s)	as originally filed	
claim(s)	filed with your letter of	
claim(s)	filed with your letter of	
claim(s)	filed with your letter of	
<input type="checkbox"/> drawings, sheeting.	as originally filed	
drawings, sheeting.	filed with your letter of	
b. <input type="checkbox"/> The amendments resulted in the cancellation of the following sheets: .....		
c. <input type="checkbox"/> This report has been established as if the amendments indicated on the extra sheet have not been made, since, for the reasons indicated, they have been considered to go beyond the disclosure as filed.		
2. PRIORITY <sup>*</sup>		
a. This report has been established as if no priority has been claimed due to the failure to furnish within the prescribed time limit the requested:		
<input type="checkbox"/> copy of the earlier application whose priority has been claimed.		
<input type="checkbox"/> translation of the earlier application whose priority has been claimed.		
b. <input type="checkbox"/> This report has been established as if no priority has been claimed due to the fact that the priority claim has been found invalid.		
Thus, for the purposes of this report, the international filing date indicated above is considered to be the relevant date.		
<sup>*</sup> Where replacement sheets are annexed to this report, a translation of these replacement sheets must be furnished to the elected Offices within the time limit applicable under PCT Article 38(1).		

**CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all.)**

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC<sup>4</sup>: G01N 33/543 G01N 33/78 33/76

**REASONED STATEMENT AS TO CLAIMS MEETING CRITERIA OF NOVELTY (N), INVENTIVE STEP (IS)  
AND INDUSTRIAL APPLICABILITY (IA), AND CITATIONS<sup>7</sup> AND EXPLANATIONS<sup>8</sup>  
SUPPORTING SUCH STATEMENT**

CLAIM NUMBER	STATEMENT (CRITERIA)	CITATIONS AND EXPLANATIONS
1-11	Yes(N, IS, IA)	<p>All claims meet the requirements of novelty, inventive step and industrial application. W088/01058, 84/01031 and GB 2099578 are acknowledged in the specification as prior art which does not teach or lead towards the specific V/K ratio of the invention.</p> <p>GB 2030290 and W086/01604 are merely illustrative of solid phase immunoassays. Clin. Chem is concerned with a specific mathematical treatment of the T4 binding system and does not lead towards the present invention.</p>



PCT/DTG 20 DEC 1988  
 PATENT COOPERATION TREATY 1988  
 INTERNATIONAL SEARCH REPORT

DCT 89

<b>IDENTIFICATION OF INTERNATIONAL APPLICATION</b>		Applicant's or Agent's File Reference SGH/Case 9
International Application No. PCT/GB 88/00649	International Filing Date 5th August 1988	
Receiving Office RO/GB	Priority Date Claimed 10th February 1988 6th August 1987	
Applicant EKINS, Roger Philip		
I. <input type="checkbox"/> CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup> (Observations on supplemental sheet (2))		
II. <input type="checkbox"/> UNITY OF INVENTION IS LACKING <sup>2</sup> (Observations on supplemental sheet (2))		
<b>III. TITLE, ABSTRACT AND FIGURE OF DRAWING</b>		
1. The following indicated items are approved as submitted by the applicant: <sup>3</sup> <div style="display: flex; justify-content: space-around;"> <span><input checked="" type="checkbox"/> Title.</span> <span><input checked="" type="checkbox"/> Abstract.</span> </div>		
2. The texts established by this International Searching Authority of the following indicated items are set forth below: <div style="display: flex; justify-content: space-around;"> <span><input type="checkbox"/> Title.</span> <span><input type="checkbox"/> Abstract.</span> </div>		
<input type="checkbox"/> Text of the abstract continued on supplemental sheet (1)		
3. a. <input type="checkbox"/> The definitive contents of the abstract are established by this International Searching Authority as proposed in form PCT/ISA/204 previously sent to the applicant.		
b. <input type="checkbox"/> This report is incomplete as far as the abstract is concerned as the time limit for comments by the applicant on the draft prepared by this International Searching Authority has not expired. <sup>4</sup>		
4. Figure to be published with the abstract: <sup>5</sup> Figure No. _____ <input checked="" type="checkbox"/> None of the figures <input type="checkbox"/> as suggested by the applicant <input type="checkbox"/> because the applicant failed to suggest a figure <input type="checkbox"/> because this figure better characterizes the invention		

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 88/00649

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>4</sup> : G 01 N 33/543; // G 01 N 33/78; G 01 N 33/76		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>4</sup>	G 01 N 33/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>8</sup></b>		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X,P	WO, A, 88/01058 (R.P. EKINS) 11 February 1988 see pages 20-25; examples 1-4; claims 1-12 cited in the application --	1,3-11
Y	WO, A, 84/01031 (R.P. EKINS) 15 March 1984 see the whole document	1
A	cited in the application --	2-9
Y	EP, A, 0063810 (CIBA-GEIGY AG) 3 November 1982 see pages 32-41; examples 2-5; claim 1	1
A	& GB, A, 2099578 (cited in the application) --	7-10
A	Clinical Chemistry, vol. 31, no. 10, October 1985 (Washington, DC, US) T.A. Wilkins et al.: "Comprehensive study of a thyroxin-analog-based assay for free thyroxin ("Amerlex FT4")	1-9  ./.
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 21st October 1988	Date of Mailing of this International Search Report 29. 11. 88	
International Searching Authority  EUROPEAN PATENT OFFICE	Signature of Authorized Officer  <b>P.C.G. VAN DER PUTTEN</b>	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 1644-1653, see page 1645, column 1; page 1649, column 1 --	
A	GB, A, 2030290 (BAXTER TRAVENOL LABORA- TORIES INC.) 2 April 1980 see page 3, lines 13-41; claims 1-26 --	1,5-9
A	WO, A, 86/01604 (R.P. EKINS) 13 March 1986 see claims 12-19 -----	1,5-9

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 8800649  
SA 23695

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 23/11/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8801058	11-02-88	AU-A- 7755987	24-02-88
		EP-A- 0271974	22-06-88
WO-A- 8401031	15-03-84	AU-A- 1944283	29-03-84
		EP-A- 0134215	20-03-85
EP-A- 0063810	03-11-82	GB-A, B 2099578	08-12-82
		JP-A- 58009070	19-01-83
		AU-A- 8306982	04-11-82
		CA-A- 1200761	18-02-86
		AU-B- 560790	16-04-87
GB-A- 2030290	02-04-80	LU-A- 81619	07-12-79
		BE-A- 878687	31-12-79
		NL-A- 7906584	14-03-80
		FR-A, B 2436396	11-04-80
		DE-A, C 2936307	10-04-80
		AU-A- 4857579	20-03-80
		JP-A- 55039089	18-03-80
		US-A- 4292296	29-09-81
		CA-A- 1113391	01-12-81
		AU-B- 524202	02-09-82
		SE-A- 7907535	13-03-80
		SE-B- 450296	15-06-87
WO-A- 8601604	13-03-86	AU-A- 4724185	24-03-86
		EP-A- 0222766	27-05-87
		JP-T- 62501442	11-06-87



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>4</sup> :</b> <b>G01N 33/543 // G01N 33/78</b> <b>G01N 33/76</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 89/ 01157</b> <b>(43) International Publication Date:</b> 9 February 1989 (09.02.89)
<b>(21) International Application Number:</b> PCT/GB88/00649 <b>(22) International Filing Date:</b> 5 August 1988 (05.08.88) <b>(31) Priority Application Numbers:</b> 8700558 8803000 <b>(32) Priority Dates:</b> 6 August 1987 (06.08.87) 10 February 1988 (10.02.88) <b>(33) Priority Country:</b> GB <b>(71)(72) Applicant and Inventor:</b> EKINS, Roger, Philip [GB/ GB]; Department of Molecular Endocrinology, Mid- dlesex Hospital Medical School, Mortimer Street, London W1N 8AA (GB). <b>(74) Agent:</b> HALE, Stephen, Geoffrey; J.Y. & G.W. John- son, Furnival House, 14-18 High Holborn, London WC1V 6DE (GB).		<b>(81) Designated States:</b> AT, AT (European patent), AU, BE (European patent), BR, CH, CH (European patent), DE, DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), HU, IT (Euro- pean patent), JP, KR, LU (European patent), NL, NL (European patent), NO, SE, SE (European patent), SU, US.  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt</i> <i>of amendments.</i>
<b>(54) Title:</b> DETERMINATION OF AMBIENT CONCENTRATION OF SEVERAL ANALYTES		
<b>(57) Abstract</b>  <p>A method for determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V litres, comprises loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart locations such that each location has not more than 0.1 V/K, preferably less than 0.01 V/K, moles of a single binding agent, where K litres/mole is the equilibrium constant of the binding agent for the analyte; contacting the loaded support means with the liquid sample to be analysed, such that each of the spaced apart locations is contacted in the same operation with the liquid sample, the amount of liquid used in the sample being such that only an insignificant proportion of any analyte present in the liquid sample becomes bound to the binding agent specific for it, and measuring a parameter representative of the fractional occupancy by the analytes of the binding agents at the spaced apart locations by a competitive or non-competitive assay technique using a site-recognition reagent for each binding agent capable of recognising either the unfilled binding sites or the filled binding sites on the binding agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent in the particular location to be measured. A device and kit for use in the method are also provided.</p>		

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Determination of ambient concentrations of  
several analytes

Field of the invention

The present invention relates to the determination of  
5 ambient analyte concentrations in liquids, for example the  
determination of analytes such as hormones, proteins and  
other naturally occurring or artificially present substan-  
ces in biological liquids such as body fluids.

Background of the invention

10 I have proposed in International Patent Application  
WO84/01031 to measure the concentration of an analyte in a  
fluid by contacting the fluid with a trace amount of a  
binding agent such as an antibody specific for the analyte  
in the sense that it reversibly binds the analyte but not  
15 other components of the fluid, determining a quantity  
representative of the proportional occupancy of binding  
sites on the binding agent and estimating from that  
quantity the analyte concentration. In that application I  
point out that, provided that the amount of binding agent  
20 is sufficiently low that its introduction into the fluid  
causes no significant diminution of the concentration of  
ambient (unbound) analyte, the fractional occupancy of the  
binding sites on the binding agent by the analyte is  
effectively independent of the absolute volume of the fluid  
25 and of the absolute amount of binding agent, i.e. indepen-  
dent within the limits of error usually associated with the  
measurement of fractional occupancy. In such circumstan-  
ces, and in these circumstances only, the initial con-  
centration  $[H]$  of analyte in the fluid is related to the  
30 fraction  $(Ab/Ab_0)$  of binding sites on the binding agent  
occupied by the analyte by the equation:

2

$$\frac{Ab}{Ab_0} = \frac{K_{ab}[H]}{1 + K_{ab}[H]}$$

where  $K_{ab}$  (hereinafter referred to as  $K$ ) is the equilibrium constant for the binding of the analyte to the binding sites and is a constant for a given analyte and binding agent at any one temperature. This constant is generally known as the affinity constant, especially when the binding agent is an antibody, for example a monoclonal antibody.

The concept of using only a trace amount of binding agent is contrary to generally recommended practice in the field of immunoassay and immunometric techniques. For example, in such a well-known work as "Methods in Investigative and Diagnostic Endocrinology", ed. S.A. Berson and R.S. Yalow, 1973 at pages 111-116, it is proposed that in the performance of a competitive immunoassay maximum sensitivity of the assay is achieved if the proportion of the "tracer" analyte that is bound approximates to 50%. In order to achieve such a high degree of binding of the analyte the theory of Berson and Yalow, to this day generally accepted by other workers in the field, requires that the concentration of binding agent (or, strictly speaking, of binding sites, each molecule of binding agent conventionally having one or at most two binding sites) must be greater than or equal to the reciprocal of the equilibrium constant ( $K$ ) of the binding agent for the analyte, i.e.  $[Ab] \geq 1/K$ . For a sample of volume  $V$  the total amount of binding agent (or binding sites) must therefore be greater than or equal to  $V/K$ . A binding agent which is a monoclonal antibody may, for example, have an equilibrium constant ( $K$ ) which is of the order of  $10^{11}$  litres/mole for the specific antigen to which it binds. Thus, under the above generally accepted practice, a binding agent (or site) concentration of the order of  $10^{-11}$  mole/litre or more is required for binding agents of such an equilibrium constant and, with fluid sample volumes of

the order of 1 millilitre, the use of  $10^{-14}$  or more mole of binding agent (or site) is conventionally deemed necessary. Avogadro's number is about  $6 \times 10^{23}$  so that  $10^{-14}$  mole of binding site is equivalent to more than  $10^9$  molecules of binding agent even assuming that the binding agent possesses two binding sites per molecule. For specific binding agents of the very highest affinity  $K$  is less than  $10^{13}$  litres/mole so that conventional practice requires more than  $10^7$  molecules of binding agent, whereas binding agents with lower affinity of the order of  $10^8$  litres/mole necessitate the use of more than  $10^{12}$  molecules under conventional practice. In fact all immunoassay kits marketed commercially at the present time conform to these concepts and use an amount of binding site approximating to or, more frequently, considerably in excess of  $V/K$ ; indeed in certain types of kit relying on the use of labelled antibodies it is conventional to use as much binding agent as possible, binding proportions of analyte greatly exceeding 50%.

Because of the binding of substantial proportions, for example 50%, of the analyte in the liquid samples under test in such systems, the fractional occupancy of the binding sites of the binding agent is not independent of the volume of the fluid sample so that for accurate quantitative assays it is necessary to control accurately the volume of the sample, keeping it constant in all tests, whether of the sample of unknown concentration or of the standard samples of known concentration used to generate the dose response curve. Furthermore, such systems also require careful control of the amount of binding agent present in the standard and control incubation tubes. These limitations of present techniques are universally recognised and accepted.

UK Patent Application 2,099,578A discloses a device for immunoassays comprising a porous solid support to which antigens, or less frequently immunoglobulins, are bound at

a plurality of spaced apart locations, said device permitting a large number of qualitative or quantitative immunoassays to be performed on the same support, for example to establish an antibody profile of a sample of human blood serum. However, although the individual locations may be in the form of so-called microdots produced by supplying droplets of antigen-containing solutions or suspensions, the number of moles of antigen present at each location is apparently still envisaged as being enough to bind essentially all of the analyte (e.g. antibody) whose concentration is to be measured that is present in the liquid sample under test. This is apparent from the fact that the quantitative method used in that application (page 3, lines 21-28) involves calibration with known amounts of immunoglobulin being applied to the support; but this means that, in the samples being tested, essentially every molecule must be extracted from the sample in order for a true comparison to be made and hence that large amounts of antigen (i.e. the binding agent in this situation) are required in each microdot, greatly in excess of the total amount of analyte (i.e. antibody in this situation) present in the sample.

#### Summary of the invention

The present invention involves the realisation that the use of high quantities of binding agent is neither necessary for good sensitivity in immunoassays nor is it generally desirable. If, instead of being kept as large as possible, the amount of binding agent is reduced so that only an insignificant proportion of the analyte is reversibly bound to it, generally less than 10%, usually less than 5% and for optimum results only 1 or 2% or less, not only is it no longer necessary to use an accurately controlled, constant volume for all the liquid samples (standard solutions and unknown samples) in a given assay, but it is also possible to obtain reliable and sometimes even improved estimates of analyte concentration using much

less than  $V/K$  moles of binding agent binding sites, say not more than  $0.1 V/K$  and preferably less than  $0.01 V/K$ . For a binding agent having an equilibrium constant ( $K$ ) for the analyte of the order of  $10^{11}$  litres/mole and samples of approximately 1 ml size this is approximately equivalent to not more than  $10^8$ , preferably less than  $10^7$ , molecules of binding agent at each location in an individual array. If the value of  $K$  is  $10^{13}$  litres/mole the figures are  $10^6$  and  $10^5$  molecules respectively, and if  $K$  is of the order of  $10^8$  litres/mole they are  $10^{11}$  and  $10^{10}$  molecules respectively. Below  $10^2$  molecules of binding agent at a single location the accuracy of the measurement would become progressively less as the fractional occupancy of the binding agent sites by the analyte would be able to change only in discrete steps as individual sites become occupied or unoccupied, but in principle at least the use of as low as 10 molecules would be permissible if an estimate with an accuracy of 10% is acceptable. Practical considerations may give rise to a preference for more than  $10^4$  molecules.

I have found that, generally speaking, for antibodies having an affinity constant  $K$  litres/mole for an antigen, the relationship between the antibody concentration and the fractional occupancy of the binding sites at any particular antigen concentration and the relationship between the antibody concentration and the percentage of antigen bound to the binding sites at any particular antigen concentration follow the same curves provided that the antibody concentrations and the antigen concentrations are each expressed in terms of fractions or multiples of  $1/K$ . This is illustrated by the accompanying drawing which is a graph representing two sets of curves plotting these relationships. Each curve relates to the antibody concentration  $[Ab]$ , expressed in terms of  $1/K$ , plotted along the x-axis. For the set of curves which remain constant or decline with increasing  $[Ab]$ , the y-axis represents the fractional occupancy ( $F$ ) of binding sites on the antibody by the antigen; for the second set, the y-axis

represents the percentage (b%) of antigen bound to those binding sites. The individual curves in each set represent the relationships corresponding to four different antigen concentrations [An] expressed in terms of K, namely 10/K, 1.0/K, 0.1/K and 0.01/K. The curves show that as [Ab] falls F reaches an essentially constant level, the value of which is dependant on [An].

It will be appreciated therefore that the abovementioned GB patent application 2,099,578A, which for quantitative estimation relies on large amounts of binding agent and essentially total sequestration of all analyte, fails to recognise the advance achieved by the present invention, which instead relies on a different analytical principle requiring measurement of the fractional occupancy of the binding agent and which thus requires only a very low proportion of the total analyte molecules present to be sequestered from the sample.

Following the recognition that the use of such small amounts of binding agent is permissible, it becomes feasible to place the binding agent required for a single concentration measurement on a very small area of a solid support and hence to place in juxtaposition to one another but at spatially separate points on a single solid support a wide variety of different binding agents specific for different analytes which are or may be present simultaneously in a liquid to be analysed. Simultaneous exposure of each of the separate points to the liquid to be analysed will cause each binding agent spot to take up the analyte for which it is specific to an extent (i.e. fractional binding site occupancy) representative of the analyte concentration in the liquid, provided only that the volume of solution and the analyte concentration therein are large enough that only an insignificant fraction (generally less than 10%, usually less than 5%) of the analyte is bound to the point. The fractional binding site occupancy for each binding agent can then be determined

using separate site-recognition reagents which recognise either the unfilled binding sites or filled binding sites of the different binding agents and which are labelled with markers enabling the concentration levels of the separate reagents bound to the different binding agents to be measured, for example fluorescent markers. Such measurements may be performed consecutively, for example using a laser which scans across the support, or simultaneously, for example using a photographic plate, depending on the nature of the labels. Other imaging devices such as a television camera can also be used where appropriate. Because the binding agents are spatially separate from one another it is possible to use only a small number of different marker labels or even the same marker label throughout and to scan each binding agent location separately to determine the presence and concentration of the label. By use of the invention considerably more than 3 analyses can be performed with a single exposure of the solid support with liquid to be analysed, for example 10, 20, 30, 50 or even up to 100 or several hundreds of analyses.

Overall, therefore, the present invention provides a method for determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V litres, comprising:

loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid and is specific for that analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart locations such that each location has not more than  $0.1 V/K$  moles of a single binding agent, where K litres/mole is the equilibrium constant of the binding agent for the analyte, contacting the loaded support means with the liquid sample to be analysed such that each of the spaced apart locations is contacted in the same operation with the liquid sample, the amount of liquid used in the sample

being such that only an insignificant proportion of any analyte present in the liquid sample becomes bound to the binding agent specific for it, and

measuring a parameter representative of the fractional  
5 occupancy by the analytes of the binding agents at the spaced apart locations by a competitive or non-competitive assay technique using a site-recognition reagent for each binding agent capable of recognising either the unfilled binding sites or the filled binding sites on the binding  
10 agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent in the particular location to be measured.

The invention also provides a device for use in determining the ambient concentrations of a plurality of  
15 analytes in a liquid sample of volume  $V$  litres, comprising a solid support means having located thereon at a plurality of spaced apart locations a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in the liquid  
20 sample and is specific for that analyte as compared to the other components of the liquid sample, each location having not more than  $0.1 V/K$ , preferably less than  $0.01 V/K$ , moles of a single binding agent, where  $K$  litres/mole is the equilibrium constant of that binding agent for reaction  
25 with the analyte to which it is specific.

A kit for use in the method according to the invention comprises a device according to the invention, a plurality of standard samples containing known concentrations of the analytes whose concentrations in the liquid sample are to  
30 be measured and a set of labelled site-recognition reagents for reaction with filled or unfilled binding sites on the binding agents.

#### Detailed description

The choice of a solid support is a matter to be left

to the user. Preferably the support is non-porous so that the binding agent is disposed on its surface, for example as a monolayer. Use of a porous support may cause the binding agent, depending on its molecular size, to be  
5 carried down into the pores of the support where its exposure to the analyte whose concentration is to be determined may likewise be affected by the geometry of the pores, so that a false reading may be obtained. Porous supports such as nitrocellulose paper dotted with spots of  
10 binding agent are therefore less preferred. Unlike the supports used in GB 2,099,578A, which seem to need to be porous because of the large number of molecules to be attached, the supports for use in the present invention use much smaller quantities and therefore need not be  
15 porous. The non-porous supports may, for example be of plastics material or glass, and any convenient rigid plastics material may be used. Polystyrene is a preferred plastics material, although other polyolefins or acrylic or vinyl polymers could likewise be used.

20 The support means may comprise microbeads, e.g. of such a plastics material, which can be coated with uniform layers of binding agent and retained in specified locations, e.g. hollows, on a support plate. Alternatively the material may be in the form of a sheet or plate which is  
25 spotted with an array of dots of binding agent. It can be advantageous for the configuration of the support means to be such that liquid samples of approximately the volume V litres are readily retained in contact with the plurality of spaced apart locations marked with the different binding  
30 agents. For example, the spaced apart locations may be arranged in a well in the support means, and a plurality of wells, each provided with the same group of different binding agents in spaced apart locations, can be linked together to form a microtitre plate for use with a  
35 plurality of samples.

When the support means is to be used in conjunction

with a measuring system involving light scanning, the material, e.g. plastics, for the support is desirably opaque to light, for example it may be filled with an opacifying material which may inter alia be white or black, such as carbon black, when the signals to be measured from the binding agent or the site-recognition reagent are light signals, as from fluorescent or luminescent markers. In general, reflective materials are preferred in this case to enhance light collection in the detecting instrument or photographic plate. The final choice of optimum material is governed by its ability to attach the binding agent to its surface, its absence of background signal emission and its possession of other properties tending to maximise the signal/noise ratio for the particular marker or markers attached to the binding agent situated on its surface. Very satisfactory results have been obtained in the Examples described below by the use of a white opaque polystyrene microtitre plate commercially available from Dynatech under the trade name White Microfluor microtitre wells.

The binding agents used may be binding agents of different specificity, that is to say agents which are specific to different analytes, or two or more of them may be binding agents of the same specificity but of different affinity, that is to say agents which are specific to the same analyte but have different equilibrium constants  $K$  for reaction with it. The latter alternative is particularly useful where the concentration of analyte to be assayed in the unknown sample can vary over considerable ranges, for example 2 or 3 orders of magnitude, as in the case of HCG measurement in urine of pregnant women, where it can vary from 0.1 to 100 or more IU/ml.

The binding agents used will preferably be antibodies, more preferably monoclonal antibodies. Monoclonal antibodies to a wide variety of ingredients of biological fluids are commercially available or may be made by known

techniques. The antibodies used may display conventional affinity constants, for example from  $10^8$  or  $10^9$  litres/mole upwards, e.g. of the order of  $10^{10}$  or  $10^{11}$  litres/mole, but high affinity antibodies with affinity constants of  $10^{12}$ -  
5  $10^{13}$  litres/mole can also be used. The invention can be used with such binding agents which are not themselves labelled. However, it is also possible and frequently desirable to use labelled binding agents so that the system binding agent/analyte/site-recognition reagent  
10 includes two different labels of the same type, e.g. fluorescent, chemiluminescent, enzyme or radioisotopic, one on the binding agent and one on the site-recognition reagent. The measuring operation then measures the ratio of the intensity of the two signals and thus eliminates the  
15 need to place the same amount of labelled binding agent on the support when measuring signals from standard samples for calibration purposes as when measuring signals from the unknown samples. Because the system depends solely on measurement of a ratio representative of binding site  
20 occupancy, there is also no need to measure the signal from the entire spot but scanning only a portion is sufficient. Each binding agent is preferably labelled with the same label but different labels can be used.

The binding agents may be applied to the support in  
25 any of the ways known or conventionally used for coating binding agents onto supports such as tubes, for example by contacting each spaced apart location on the support with a solution of the binding agent in the form of a small drop, e.g. 0.5 microlitre, on a  $1\text{ mm}^2$  spot, and allowing them to  
30 remain in contact for a period of time before washing the drops away. A roughly constant small fraction of the binding agent present in the drop becomes adsorbed onto the support as a result of this procedure. It is to be noted that the coating density of binding agent on the microspot  
35 does not need to be less than the coating density in conventional antibody-coated tubes; the reduction in the number of molecules on each spot may be achieved solely by

reduction of the size of the spot rather than the coating density. A high coating density is generally desirable to maximise signal/noise ratios. The sizes of the spots are advantageously less than  $10 \text{ mm}^2$ , preferably less than  $1 \text{ mm}^2$ . The separation is desirably, but not necessarily, 2 or 3 times the radius of the spot, or more. These suggested geometries can nevertheless be changed as required, being subject solely to the limitations on the number of binding agent molecules in each spot, the minimum volume of the sample to which the array of spots will be exposed and the means locally available for conveniently preparing an array of spots in the manner described.

Once the binding agents have been coated onto the support it is conventional practice to wash the support, in the case of antibodies as binding agents, with a solution containing albumen or other protein to saturate all remaining non-specific adsorption sites on the support and elsewhere. To confirm that the amount of binding agent in an individual spot will be less than the maximum amount ( $0.1 \text{ V/K}$ ) required to conform to the principle of the present invention, the amount of binding agent present on any individual site can be checked by labelling the binding agent with a detectable marker of known specific activity (i.e. known amount of marker per unit weight of binding agent) and measuring the amount of marker present. Thus, if the use of labelled binder is not desired on the solid support used in the method of the invention the binding agent can nevertheless be labelled in a trial experiment and identical conditions to those found in that trial to give rise to correct loadings of binding agent can be used to apply unlabelled binding agent to the supports to be actually used.

The minimum size of the liquid sample ( $V$  litres) is correlated with the number of mole of binding agent (less than  $0.1 \text{ V/K}$ ) so that only an insignificant proportion of the analyte present in the liquid sample becomes bound to

the binding agent. This proportion is as a general rule less than 10%, usually less than 5% and desirably 1 or 2% or less, depending on the accuracy desired for the assay (greater accuracy being obtained, other things being equal, when smaller proportions of analyte are bound) and the magnitude of other error-introducing factors present. Sample sizes of the order of one or a few ml or less, e.g. down to 100 microlitres or less, are often preferred, but circumstances may arise when larger volumes are more conveniently assayed, and the geometry may be adjusted accordingly. The sample may be used at its natural concentration level or if desired it may be diluted to a known extent.

The site-recognition reagents used in the method according to the invention may themselves be antibodies, e.g. monoclonal antibodies, and may be anti-idiotypic or anti-analyte antibodies, the latter recognising occupied sites. Alternatively, for example for analytes of small molecular size such as thyroxine (T4), unoccupied sites may be recognised using either the analyte itself, appropriately labelled, or the analyte covalently coupled to another molecule - e.g. a protein molecule - which is directly or indirectly labelled. The site-recognition reagents may be labelled directly or indirectly with conventional fluorescent labels such as fluorescein, rhodamine or Texas Red or materials usable in time-resolved pulsed fluorescence such as europium and other lanthanide chelates, in a conventional manner. Other labels such as chemiluminescent, enzyme or radioisotopic labels may be used if appropriate. Each site-recognition reagent is preferably labelled with the same label but different labels can be used in different reagents. The site-recognition reagents may be specific for a single one of the binding agent/analyte spots in each group of spots or in certain circumstances, as with glycoprotein hormones such as HCG and FSH which have a common binding site, they may be cross-reacting reagents able to react with occupied binding sites in more than one

of the spots.

In the assay technique the signals representative of the fractional occupancy of the binding agent in the test samples of unknown concentrations of the analytes can be calibrated by reference to dose response curves obtained from standard samples containing known concentrations of the same analytes. Such standard samples need not contain all the analytes together, provided that each of the analytes is present in some of the standard samples. Fractional occupancy may be measured by estimating occupied binding sites (as with an anti-analyte antibody) or unoccupied binding sites (as with an anti-idiotypic antibody), as one is the converse of the other. For greater accuracy it is desirable to measure the fraction which is closer to zero because a change in fractional occupancy of 0.01 is proportionately greater in this case, although for fractional occupancies in the range 25-75% either alternative is generally satisfactory.

In that embodiment of the present invention which relies on two fluorescent markers, the measurement of relative intensity of the signals from the two markers, one on the binding agent and the other on the site recognition reagent, may be carried out by a laser scanning confocal microscope such as a Bio-Rad Lasersharp MRC 500, available from Bio-Rad Laboratories Ltd., and having a dual channel detection system. This instrument relies on a laser beam to scan the dots or the like on the support to cause fluorescence of the markers and wavelength filters to distinguish and measure the amounts of fluorescence emitted. Time-resolved fluorescence methods may also be used. Interference (so-called crosstalk) between the two channels can be compensated for by standard corrections if it occurs or conventional efforts can be made to reduce it. Discrimination of the two fluorescent signals emitted by the dual-labelled spots is accomplished in the present form of this instrument, by filters capable of distinguishing

the characteristic wavelength of the two fluorescent emissions; however, fluorescent substances may be distinguished by other physical characteristics such as differing fluorescence decay times, bleaching times, etc., and any of these means may be used, either alone or in combination, to differentiate between two fluorophores and hence permit measurement of the ratio of two fluorescent labelled entities (binding agent and site-recognition reagent) present on an individual spot, using techniques well known in the fluorescence measurement field. When only one fluorescent label is present the same techniques may be used, provided that care is taken to scan the entire spot in each case and the spots contain essentially the same amount of binding agent from one assay to the next when the unknown and standard samples are used.

In the case of other labels, such as radioisotopic labels, chemiluminescent labels or enzyme labels, analogous means of distinguishing the individual signals from one or from each of a pair of such labels are also well known. For example two radioisotopes such as  $^{125}\text{I}$  and  $^{131}\text{I}$  may be readily distinguished on the basis of the differing energies of their respective radioactive emissions. Likewise it is possible to identify the products of two enzyme reactions, deriving from dual enzyme-labelled antibody couplets, these being e.g. of different colours, or two chemiluminescent reactions, e.g. of different chemiluminescent lifetime or wavelength of light emission, by techniques well known in the respective fields.

The invention may be used for the assaying of analytes present in biological fluids, for example human body fluids such as blood, serum, saliva or urine. They may be used for the assaying of a wide variety of hormones, proteins, enzymes or other analytes which are either present naturally in the liquid sample or may be present artificially such as drugs, poisons or the like.

For example, the invention may be used to provide a device for quantitatively assaying a variety of hormones relating to pregnancy and reproduction, such as FSH, LH, HCG, prolactin and steroid hormones (e.g. progesterone, estradiol, testosterone and androstene-dione), or hormones of the adrenal pituitary axis, such as cortisol, ACTH and aldosterone, or thyroid-related hormones, such as T4, T3, and TSH and their binding protein TBG, or viruses such as hepatitis, AIDS or herpes virus, or bacteria, such as staphylococci, streptococci, pneumococci, gonococci and enterococci, or tumour-related peptides such as AFP or CEA, or drugs such as those banned as illicit improvers of athletes' performance, or food contaminants. In each case the binding agents used will be specific for the analytes to be assayed (as compared with others in the sample) and may be monoclonal antibodies therefor.

Further details on the methodology are to be found in my International Patent Publication W088/01058, the contents of which are incorporated herein by reference.

The invention is illustrated by the following Examples.

#### Example 1

An anti-TNF (tumour necrosis factor) antibody having an affinity constant for TNF at 25°C of about  $1 \times 10^9$  litres/mole is labelled with Texas Red. A solution of the antibody at a concentration of 80 micrograms/ml is formed and 0.5 microlitre aliquots of this solution are added in the form of droplets one to each well of a Dynatech Microfluor (opaque white) filled polystyrene microtitre plate having 12 wells.

An anti-HCG (human chorionic gonadotropin) antibody having an affinity constant for HCG at 25°C of about  $6 \times 10^8$  litres/mole is also labelled with Texas Red. A

solution of the antibody at a concentration of 80 micrograms/ml is formed and 0.5 microlitre aliquots of this solution are added in the form of droplets one to each well of the same Dynatech Microfluor microtitre plate.

- 5 After addition of the droplets the plate is left for a few hours in a humid atmosphere to prevent evaporation of the droplets. During this time some of the antibody molecules in the droplets become adsorbed onto the plate. Next, the wells are washed several times with a phosphate  
10 buffer and then they are filled with about 400 microlitres of a 1% albumen solution and left for several hours to saturate the residual binding sites in the wells. Thereafter they are washed again with phosphate buffer.

- The resulting plate has in each of its wells two spots  
15 each of area approximately  $1 \text{ mm}^2$ . Measurement of the amount of fluorescence shows that in each well one spot contains about  $5 \times 10^9$  molecules of anti-TNF antibody and the other contains about  $5 \times 10^9$  molecules of anti-HCG antibody. The wells are designed for use with liquid  
20 samples of volume 400 microlitres, so that  $0.1 \text{ V/K}$  is  $4 \times 10^{-14}$  moles (equivalent to  $2.4 \times 10^{10}$  molecules) for the anti-TNF antibody and  $7 \times 10^{-14}$  moles (equivalent to  $4 \times 10^{10}$  molecules) for the anti-HCG antibody.

### Example 2

- 25 A microtitre plate prepared as described in Example 1 is used in an assay for an artificially produced solution containing TNF and HCG. A test sample of the solution, amounting to about 400 microlitres, is added to one of the wells and allowed to incubate for several hours. About 400  
30 microlitres of various standard solutions containing known concentrations (0.02, 0.2, 2 and 20 ng/ml) of TNF or HCG are added to other wells of the plate and also allowed to incubate for several hours. The wells are then washed several times with buffer solution.

As site-recognition reagents there are used for the TNF spots an anti-TNF antibody having an affinity constant for TNF at 25°C of about  $1 \times 10^{10}$  litres/mole and for the HCG spots an anti-HCG antibody having an affinity constant for HCG at 25°C of about  $1 \times 10^{11}$  litres/mole. Both antibodies are labelled with fluorescein (FITC). 400 microlitre aliquots of solutions of these labelled antibodies are added to the wells and allowed to stand for a few hours. The wells are then washed with buffer.

The resulting fluorescence ratio of each spot is quantified with a Bio-Rad Lasersharp MRC 500 confocal microscope. From the standard solutions dose response curves for TNF and HCG are built up, the figures for TNF being as follows:

15	TNF concentration ng/ml	FITC fluorescence	on TNF spot
		<hr/> Texas Red fluorescence	
20	0.02	1.1	
	0.2	4.6	
	2	7.9	
	20	42.5	

and those for HCG being as follows:

25	HCG concentration	FITC fluorescence	on HCG spot
	ng/ml	<hr/> Texas Red fluorescence	
	0.02	1.8	
	0.2	7.2	
	2	16.0	
	20	28.2	

The artificially produced solution was found to give ratio readings of 5.9 on the TNF spot and 10.5 on the HCG spot, correlating well with the actual concentrations of

TNF (0.5 ng/ml) and HCG (0.5 ng/ml) obtained from the dose response curves.

### Example 3

Using similar procedures to those outlined in Example 1 a microtitre plate containing spots of labelled anti-T4 (thyroxine) antibody (affinity constant about  $1 \times 10^{11}$  litres/mole at 25°C), labelled anti-TSH (thyroid stimulating hormone) antibody (affinity constant about  $5 \times 10^9$  litres/mole at 25°C) and labelled anti-T3 (triiodothyronine) antibody (affinity constant about  $1 \times 10^{11}$  litres/mole at 25°C) in each of the individual wells is produced, the spots containing less than  $1 \times 10^{-12}$  V moles of anti-T4 antibody or less than  $2 \times 10^{-11}$  V moles of anti-TSH antibody or less than  $1 \times 10^{-12}$  V moles of anti-T3 antibody.

The developing antibody (site-recognition reagent) for the TSH assay is an anti-TSH antibody with an affinity constant for TSH of  $2 \times 10^{10}$  litres/mole at 25°C. This antibody is labelled with fluorescein (FITC). The site-recognition reagents for the T4 and T3 assays are T4 and T3 coupled to poly-lysine and labelled with FITC, and they recognise the unfilled sites on their respective first antibodies.

Using 400 microlitre aliquots of standard solutions containing various known amounts of T4, T3 and TSH, dose response curves are obtained by methods analogous to those in Example 2, correlating fluorescence ratios with T4, T3 and TSH concentrations. The plate is used to measure T4, T3 and TSH levels in serum from human patients with good correlation with the results obtained by other methods.

### Example 4

Using similar procedures to those outlined in Example

1 a microtitre plate containing spots of first labelled  
anti-HCG antibody (affinity constant about  $6 \times 10^8$   
litres/mole at  $25^\circ\text{C}$ ), second labelled anti-HCG antibody  
(affinity constant about  $1.3 \times 10^{11}$  litres/mole at  $25^\circ\text{C}$ )  
5 and labelled anti-FSH (follicle stimulating hormone)  
antibody (affinity constant about  $1.3 \times 10^8$  litres/mole at  
 $25^\circ\text{C}$ ) in each of the individual wells is produced, the  
spots each containing less than  $0.1 \text{ V/K}$  moles of the  
respective antibody. A cross-reacting (alpha subunit)  
10 monoclonal antibody 8D10 with an affinity constant of  $1 \times$   
 $10^{11}$  litres/mole is used as a common developing antibody  
for both the HCG and the FSH assays.

Using 400 microlitre aliquots of standard solutions  
containing various known concentrations of HCG and FSH,  
15 dose response curves are obtained by methods analogous to  
those in Example 2, correlating fluorescence ratios with  
HCG and FSH concentrations, the curve obtained with the  
higher affinity anti-HCG antibody giving more concentra-  
tion-sensitive results at the lower HCG concentrations  
20 whereas the curve from the lower affinity anti-HCG antibody  
is more concentration-sensitive at the higher HCG con-  
centrations. The plate is used to measure HCG and FSH  
concentrations in the urine of women in pregnancy testing,  
giving good correlations with results obtained by other  
25 means and achieving effective concentration measurements  
for HCG over a concentration range of two or three orders  
of magnitude by correct choice of the best HCG spot and  
dose response curve.

#### Production of labelled antibodies

30 The labelling of the antibodies with fluorescent  
labels can be carried out by a well known and standard  
technique, see Leslie Hudson and Frank C. Hay, "Practical  
Immunology", Blackwell Scientific Publications (1980),  
pages 11-13, for example as follows:

The monoclonal antibody anti-FSH 3G3, an FSH specific (beta subunit) antibody having an affinity constant (K) of  $1.3 \times 10^8$  litres per mole, was produced in the Middlesex Hospital Medical School, and was labelled with TRITC (rhodamine isothiocyanate) or Texas Red, giving a red fluorescence.

The monoclonal antibody anti-FSH 8D10, a cross-reacting (alpha subunit) antibody having an affinity constant (K) of  $1 \times 10^{11}$  litres per mole, was likewise produced in the Middlesex Hospital Medical School and was labelled with FITC (fluorescein isothiocyanate), giving a yellow-green fluorescence.

The general procedure used involved ascites fluid purification (ammonium sulphate precipitation and T-gel chromatography) followed by labelling, according to the following steps:

1.a. Ammonium sulphate purification

1. Add 4.1 ml saturated ammonium sulphate solution to 5 ml antibody preparation (culture supernatant or 1:5 diluted ascites fluid) under constant stirring (45% saturation).

2. Continue stirring for 30-90 min. Centrifuge at 2500 rpm for 30 min.

3. Discard the supernatant and dissolve the precipitate in PBS (final volume 5 ml.). Repeat Steps 1 and 2, OR

4. Add 3.6 ml saturated ammonium sulphate (40% saturation) under constant stirring. Repeat Step 2.

5. Discard the supernatant and dissolve the pellet in the desired buffer.

6. Dialyse overnight in cold against the same buffer (using fresh, boiled-in-d/w dialysis bag).

7. Determine the protein concentration either at  $A_{280}$  or by Lowry estimation.

1.b. T-gel Chromatography: (Buffer: 1M Tris-Cl, pH 7.6. Solid potassium sulphate)

1. Clear 2 ml of ascites fluid by centrifugation at 4000 rpm.

5        2. Add 1 M Tris-Cl solution to achieve final concentration of 0.1 M.

3. Add sufficient amount of solid potassium sulphate. Final concentration = 0.5 M.

4. Apply the ascite fluid to the T-gel column.

10       5. Wash the column with 0.1 M Tris-Cl buffer containing 0.5 M potassium sulphate, until protein profile (at  $A_{280}$ ) returns to zero.

6. Elute the absorbed protein using 0.1 M Tris-Cl buffer as the eluant.

15       7. Pool the fractions containing antibody activity and concentrate using Amicon 30 concentrater.

8. If HPHT purification is to be carried out, use HPHT chromatography Starting buffer during Step 7.

2. Labelling of Antibodies FITC/TRITC conjugation:

20       1. Dialyse the purified 1 g protein into 0.25 M Carbonate-bicarbonate buffer, pH 9.0 to a concentration of 20 mg/ml.

2. Add FITC/TRITC to achieve a 1:20 ratio with protein (i.e. 0.05 mg for every 1 mg of protein).

25       3. Mix and incubate at 4°C for 16-18 hrs.

4. Separate the conjugated protein from unconjugated by:

a. Sephadex G-25 chromatography for FITC label,

or b. DEAE-Sephacel chromatography for TRITC/FITC label.

30       Buffer system:

PBS for (a).

0.005 M Phosphate, pH 8.0 and 0.18 M

Phosphate, pH 8.0 for (b).

Calculation of FITC: Protein coupling ratio:-

35        $2.87 \times 0.D.495 \text{ nm}$

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$0.D.280 \text{ nm} - 0.35 \times 0.D.495 \text{ nm}$

CLAIMS

1. A method for determining the ambient concentrations of a plurality of analytes in a liquid sample of volume  $V$  litres, comprising

5 loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, onto a support means at a plurality of  
10 spaced apart locations such that each location has not more than  $0.1 V/K$  moles of a single binding agent, where  $K$  litres/mole is the equilibrium constant of the binding agent for the analyte;

contacting the loaded support means with the liquid  
15 sample to be analysed, such that each of the spaced apart locations is contacted in the same operation with the liquid sample, the amount of liquid used in the sample being such that only an insignificant proportion of any analyte present in the liquid sample becomes bound to the  
20 binding agent specific for it, and

measuring a parameter representative of the fractional occupancy by the analytes of the binding agents at the spaced apart locations by a competitive or non-competitive assay technique using a site-recognition reagent for each  
25 binding agent capable of recognising either the unfilled binding sites or the filled binding sites on the binding agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent, in the particular location to be measured.

30 2. A method as claimed in claim 1 wherein each of the spaced apart locations has less than  $0.01 V/K$  moles of a single binding agent.

3. A method as claimed in claim 1 wherein the binding agents used have equilibrium constants for the analytes of

from  $10^8$  to  $10^{13}$  litres per mole.

4. A method as claimed in claim 1 wherein the binding agents used have equilibrium constants for the analytes of the order of  $10^{10}$  or  $10^{11}$  litres per mole.

5 5. A method as claimed in claim 1 wherein the volume of the liquid sample is not more than 0.1 litre.

6. A method as claimed in claim 1 wherein the volume of the liquid sample is 400 to 1000 microlitres.

7. A method as claimed in claim 1 wherein the binding  
10 agents loaded onto the support means are antibodies for the analytes whose concentrations are to be determined.

8. A method as claimed in claim 1 wherein the binding agents are labelled with markers enabling the concentration levels of the binding agent to be measured.

15 9. A method as claimed in claim 8 wherein the binding agents and the site-recognition reagents are labelled with fluorescent markers such that at the individual spaced apart locations the assay technique for measuring fractional occupancy of the binding agents measures the ratios of  
20 the signals emitted by the fluorescent markers.

10. A device for use in determining the ambient concentrations of a plurality of analytes in a liquid sample of volume  $V$  litres, comprising a solid support means having located thereon at a plurality of spaced apart  
25 locations a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, each location having not  
30 more than  $0.1 V/K$  moles of a single binding agent, where  $K$  litres/mole is the equilibrium constant of that binding

agent for reaction with the analyte to which it is specific.

11. A kit for use in determining the ambient  
5 concentration of a plurality of analytes in a liquid sample of volume  $V$  litres, comprising

a solid support means having located thereon at a plurality of spaced apart locations a plurality of different binding agents, each binding agent being capable  
10 of reversibly binding an analyte which is or may be present in the liquid sample and is specific for that analyte as compared to the other components of the liquid sample, each location having not more than  $0.1 V/K$ , preferably less than  $0.01 V/K$ , moles of a single binding agent, where  $K$   
15 litres/mole is the equilibrium constant of that binding agent for reaction with the analyte to which it is specific,

a plurality of standard samples containing known concentrations of the analytes whose concentrations in the  
20 liquid sample are to be measured, and

a set of labelled site-recognition reagents for reaction with filled or unfilled binding sites on the binding agents.

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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	pages 1644-1653, see page 1645, column 1; page 1649, column 1	
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A	GB, A, 2030290 (BAXTER TRAVENOL LABORA- TORIES INC.) 2 April 1980 see page 3, lines 13-41; claims 1-26	1,5-9
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A	WO, A, 86/01604 (R.P. EKINS) 13 March 1986 see claims 12-19	1,5-9
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**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 8800649

SA 23695

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 23/11/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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